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REVIEW ARTICLE

Some observations on the effects of bioprocessing on biopolymer stability

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Abstract

A short review is given of some of the effects of the stresses encountered during bioprocessing of protein and carbohydrate-based macromolecular systems. This is of relevance to the effectiveness and safety of protein or peptide drugs themselves (such as insulin and monoclonal antibodies) and for the integrity of delivery systems (such as various carbohydrate-based hydrogel or mucoadhesive polymers). Some carbohydrate polymers are themselves bioactive or immunostimulatory and particular use is being made of polysaccharide and glycoconjugate vaccines whose effectiveness can be severely effected by chain degradation. Stability criteria include molecular weight and conformation and techniques ranging from simple viscomery measurements to sophisticated analytical ultracentrifuge and multi-angle light scattering coupled to size exclusion chromatography and precision viscometry measurements have been useful in this regard. We focus on some recent work on the degradation and aggregation of immunoglobulin G4-based monoclonal antibodies in response to repeated freezing and thawing and long-term storage, looking at the possible connection between conformation change and aggregation, the effects of storage conditions on the stability of chitosan mucoadhesive systems used for nasal and oral delivery. We look at the effects of sterilization conditions (thermal and irradiation) on the stability of a variety of other polysaccharides such as starches, κ-carrageenan, carboxymethylcellulose, alginate, low- and high-methoxy pectins, guar, and xyloglucans and consider the use of a relatively new method for the evaluation of the molecular weight distribution of glycoconjugate vaccines with molecular weights as high as 100×10^6 g/mol.

Keywords: Aggregation, degradation, antibodies, polysaccharides, storage, freeze-thaw, thermal, irradiation.

Introduction

In recent years the issue of macromolecular stability has gained increased importance in the biopharmaceutical industry and will continue to gain in importance with the rapid development of therapeutic protein products and carbohydrate-based delivery systems. Various aspects of bioprocessing however can impose tremendous stresses on macromolecular-based products. The stresses encountered include freezing and thawing, sterilisation (thermal processing and irradiation), spray drying, fermentation, filtration, extrusion, lyophilisation, pegylation, shipping, and storage.

With regard to protein-based products for example—ranging from insulin for the treatment of diabetes to the production of monoclonal antibodies for the treatment of tumors—the shelf-life required for commercial viability of a pharmaceutical protein product is between 18 and 24 months. However, it is difficult to achieve this because proteins are highly susceptible to a variety of degradation mechanisms that have been classified in terms of chemical and physical instability. These mechanisms can occur simultaneously and may lead to formation of either soluble or insoluble aggregates, depending on the protein, environmental conditions, and stage of the aggregation process. Protein aggregation is a particular problem in biopharmaceutical processing because it is routinely encountered during fermentation, refolding, purification, sterilisation, shipping, and storage operations (Manning et al., 1989; Cleland et al., 1993; Brange, 2000; Goolcharran et al., 2000; Chi et al., 2003; Remmele et al., 2006; Wang, 2005). Problems can be particularly acute in the production of monoclonal antibodies

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intended for therapeutic purposes. These multi-domain structures linked by a potentially fragile hinge region can experience temperature, pH and pressure events that can all lead to aggregation and in some cases fragmentation. Complexities in monoclonal antibody aggregation result from different types of stress involved in protein preparations that may lead to different intermolecular forms of aggregates. Chain degradation for large polysaccharide or glycoconjugate structures is a particular problem for both direct therapeutic applications—such as the application of carboxymethylcellulose and guar in the treatment of keratoconjunctivitis (Petricek et al., 2008)—or as hydrogels, film formers, encapsulants, and mucoadhesives for drug delivery (see e.g. Davis et al., 1986; Anderson et al., 1989; Melia, 1991; Errington et al., 1992, 1993; Fiebrig et al., 1994, 1995a, 1995b, 1997; Roberts et al., 1995; Aspden et al., 1996; He et al., 1998; Illum, 1998; Tombs and Harding, 1998; Harding et al., 1999; Deacon et al. 1999, 2000; Illum et al., 2001; Harding 2003, 2006; Fee et al., 2003; Fee, 2005; Ghong et al., 2009; Wandrey et al., 2009). It is also a particular issue for proteins that have significant linear regions of unfolded structure such as the *Mytilus edulis* (mussel) "glue protein"(Deacon et al., 1997), considered as a potential mucoadhesive (Deacon et al. 1998) and also for synthetic polymer-based delivery systems (see e.g. Hagan et al., 1996). Whether protein or saccharide based, a variable product can lead to variable properties, can invoke unwanted immunological or other metabolic responses and hence be undesirable for both the patient and the regulatory authorities.

This short review considers the consequences of bioprocessing on the physical integrity of proteins and polysaccharides directly or indirectly involved with drug targeting. It is not comprehensive and focuses on some of the principal techniques for characterizing physical stability used (molecular weight distribution, state of aggregation, conformation, and flexibility) as well as specific biological macromolecules. Chemical stability as can be assayed by for example the powerful tools of ^{1}H and ^{13}C NMR and electrospray mass spectroscopy will not be considered here. Theoretical and experimental detail is also kept to a minimum but hopefully it will provide an idea of how problems are identified providing the researcher in industry the necessary clues as to what improvements are required.

Stability of monoclonal antibodies in response to freeze thaw or storage

Monoclonal antibody production for the treatment or control of serious disease such as colonic cancer has focused on the human immunoglobulin G (IgG) subclasses IgG4 and IgG1 (because of their ability to cross-link antigen: choice between the two subclasses depends largely on whether an effector response is needed as well—IgG1 with its more significant hinge and flexibility being more effective in this regard—however the hinge tends to confer lower stability. An increasingly popular and Food and Drug Administration/patent favored method for assaying for stability in response to bioprocessing is the analytical ultracentrifuge, and in particular the sedimentation velocity method, as it is a free solution method involving no columns or membranes and no immobilization on surfaces (Scott et al., 2005): from analysis of distributions of the sedimentation coefficient, s, the extent of aggregation or fragmentation can be readily assessed.

Long-term storage and freeze thaw of IgG4

A recent published example was a study by Lu et al. (2008a) on the effect of long-term storage or repeated freeze thaw on solutions of monoclonal IgG4, namely the effect of (i) elevated temperature storage for up to 59 days at 37°C or (ii) a series of freeze thaw cycles (storage at −80°C then incubation at 20°C for 1 h under different conditons). Sedimentation coefficient distribution analysis (measured at 20°C after the treatment process had been complete) clearly indicated that although the "monomeric" IgG4 remained the dominant species, there was progressive increase in the heterogeneity or proportion of aggregation products in both cases (Figure 1): the relative proportion of the various components could be estimated by multi-Gaussian analysis of the sedimentation distribution g(s) versus s profiles, and oligomer identification could be estimated by the relation $s \sim M^{2/3}$ for globular proteins. Interestingly, the relative proportion of the higher molecular weight species did not increase with increase in loading concentration (see also Longman, 2005), indicating that the aggregates were not in reversible equilibrium with the monomeric species. Some fragmentation to yield "half antibodies" was also observed.

Lu et al. (2008a) also explored the effect of a point mutant variant of the IgG4, where a serine at residue 241 had been replaced by a proline residue. As with the wild type, solution heterogeneity was found to increase considerably with storage or freeze thaw bioprocessing, although the mutation did appear to confer more stability against freeze thaw.

Another interesting finding of the Lu et al. (2008a) study was the change in the sedimentation coefficient of the "monomer" species as the extent of aggregation increased on bioprocessing. As the sedimentation coefficient is also a measure of conformation as well as size (a more compact shape of the same molar mass having a higher value of the sedimentation coefficient) these changes seemed to indicate that a conformation change of the antibody—in terms of average orientation of the domains—may be linked with an increased tendency to aggregate.

Pegylation of antibody fragments

Finally in another study, Lu et al. (2008b) investigated the effect of pegylation—covalent attachment of polyethylene glycocl (PEG) chains, designed to increase the lifetime of monoclonal antibodies/fragments in the body—on the stability and conformation of individual

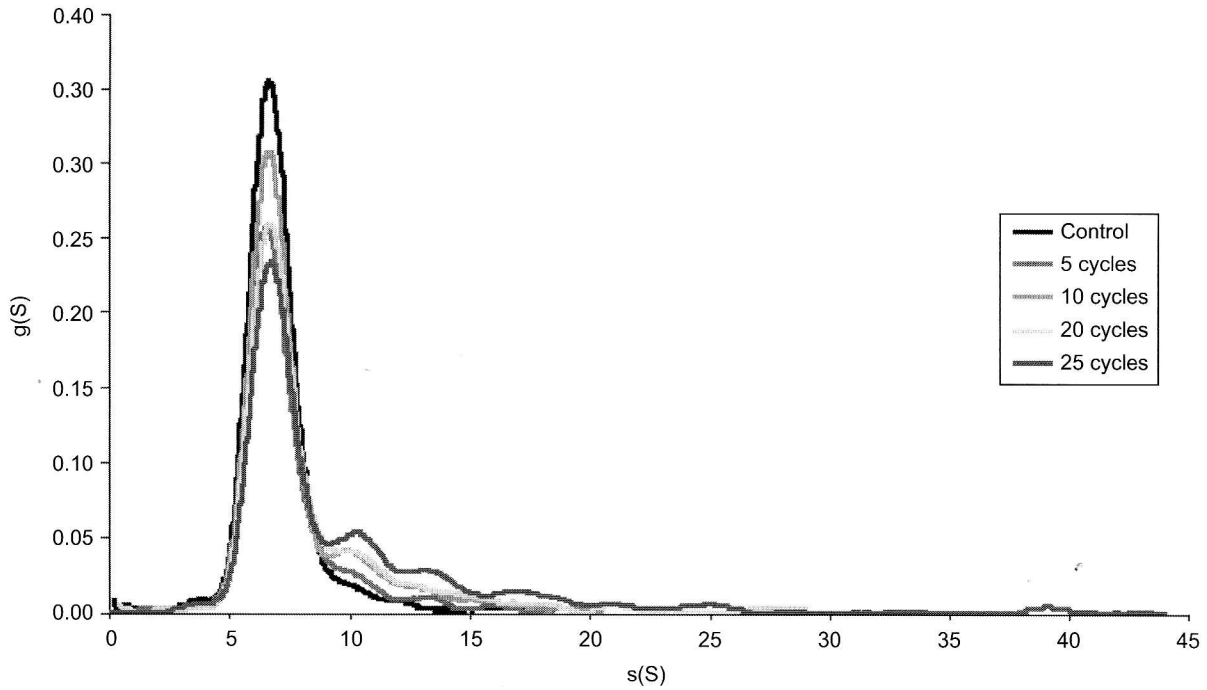

Figure 1. Effect of repeated freeze thaw on the sedimentation coefficient distribution for an IgG4 antibody that has undergone cycles of heat treatment. Loading concentrations were ~1.3 mg/ml. A reduction in the amount of the monomer species in relation to that of aggregate can be clearly seem. Adapted from Lu et al. (2008a) with permission from John Wiley and sons.

antibody fragments (human γ1 Fab′, human γ1 $F(ab')_2$; and murine γ1 Fab′) using a combination of sedimentation velocity analysis and small-angle X-ray scattering. Pegylation appeared to cause no aggregation. The sedimentation coefficient data showed that in all cases the coil like PEG chains attached dominated the hydrodynamic properties whereas ab initio modeling for the conformation of the protein component within the PEG blanket based on the small-angle X-ray solution scattering data seem to show conformation harmony with the crystal structures for the unconjugated proteins, implying the process of pegylation did not disrupt the conformation of the antibody fragments (Figure 2).

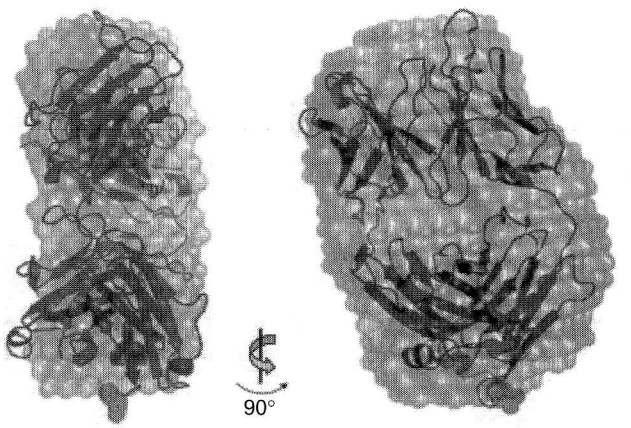

Figure 2. Ab initio modeling of SAXS data (grey spheres) for the Fab′ component of Pegylated Fab′ and the crystal structure (red, cysteine residue at position 214 indicated in green). This indicates that despite pegylation the overall conformation of the antibody fragment remains essentially the same. The hydrodynamic properties however are dominated by the attached PEG chain(s). Reproduced with permission from Lu et al. (2008b) and John Wiley and sons.

Polysaccharide stability

For polysaccharides we can use a simpler method for assaying for aggregation—or chain degradation - based on intrinsic viscosity [η] or reduced specific viscosity η_{red} measurement. The intrinsic viscosity is related to molecular weight M via a relation known as the Mark-Houwink-Kuhn Sakurada (MHKS) equation:

$$[\eta] = K'M^a \quad (1)$$

where K′ and the power-law coefficient *a* are characteristic for a macromolecule of a particular conformation type. For rigid spherical molecules the limits for *a* are 0, for random coils ~0.5–0.7 and for rod shape molecules with no flexibility the limit is $a \sim 1.8$. From these values it can be seen straight away that [η] cannot be used as a measure of molecular weight stability of proteins: a globular protein like ovalbumin (M ~ 45,000 g/mol) has an almost identical [η] (~3.4 ml/g) with the approximately spherical tomato bushy stunt virus (M ~ 10,000,000 g/mol): for these globular systems [η] is still though a very sensitive and useful measure of conformation change (e.g. protein denaturation). Most polysaccharides have conformations ranging from rigid rods to random coils and for these [η] is also very sensitive to molecular weight change.

Stability of chitosans in response to storage

This sensitivity of intrinsic viscosity (or reduced specific viscosity at a particular concentration) has been put to

particularly good use in assaying the stability of chitosans (poly N-acetyl glucosamine—chitin—deacetylated according to specification) to storage at various temperatures. Chitosans have proven the basis for the construction of efficient mucoadhesive systems for drug delivery to the small intestine and, most notably, nasal epithelia (see He et al., 1998) although concern has been expressed as to their stability, particularly as, depending on the degree of acetylation they tend to be insoluble above a pH of ~6. Fee (2005) has analysed the long-term stability/shelf-life of chitosan dispersions from measurements of η_{red} (approximated as $[\eta]$) at a concentration of 1 mg/ml and shown that storage at 4°C is desirable (Figure 3). Viscosity in conjunction with other methods has also formed the cornerstone of stability studies on other polysaccharide systems subject to stress.

Effects of thermal processing on carbohydrate polymers

Thermal processing is the most common form of sterilization technique applied to products: temperatures of 60–70°C heating for ~20 s is usually sufficient to eliminate contamination from non-sporulating bacteria that are potentially harmful to a patient. Much more severe forms of heat treatment are required for products suspected of contamination by pathogenic bacterial spores. Steam autoclaves can be used which can operate under pressure at temperatures up to 135°C. Such conditions are highly disruptive for proteins—which do not always renature correctly when the heat is removed - and with long, linear polysaccharides these conditions can lead to chain scission. Despite this there have been only a limited number of studies on the effect of thermal processing on polysaccharides, and what data we have suggests that the damage depends very much on the type of polysaccharide. Experimental protocols have either involved measurements at elevated temperature, or measurements at ~20°C after a period of thermal treatment.

Thermally processed alginate, carboxymethylcellulose and κ-carrageenan

Bradley and Mitchell (1988) used for example a specially designed slit viscometer permitting the measurement of the kinetics of degradation at temperatures over 100°C applied to alginate, carboxymethylcellulose and κ-carrageenan. The measurements were initially in terms of the zero shear viscosity from which they were able to estimate intrinsic viscosities $[\eta]$ using an approximate relation involving the coil overlap parameter. Then using the MHKS relation (eqn 1) and published values for K′ and the power-law coefficient *a* they were able to obtain approximate estimates of molecular weights M and from the slope of 1/M versus time they were able to obtain first order rate constants for the degradation. Figure 4 shows the degradation of a 15 mg/ml solution of κ-carrageenan at high temperature in terms of the molecular weight calculated in this way. From the kinetic curves and Arrhenius analysis they were able to obtain an estimate for the activation energies of depolymerisation for the three polysaccharides studied, showing that alginate was the least stable.

Thermally processed pectins

Using a completely different approach Morris et al. (1999) assessed the stability of low methoxy (LM) pectins up to a temperature of measurement of 60°C, using measurement of reduced specific viscosity η_{red} (approximated as ~$[\eta]$), together with direct measurement of the weight average molecular weight M_w from sedimentation equilibrium measurements in an analytical ultracentrifuge specially adapted to allow elevated temperature measurement. The same instrument was also use to measure sedimentation coefficients. These researchers found that

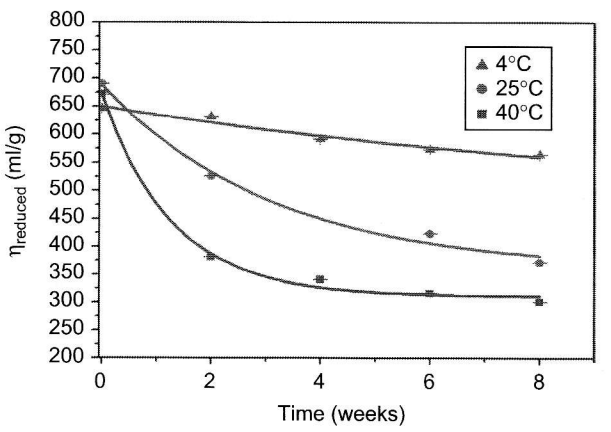

Figure 3. Intrinsic viscosity $[\eta]$ or reduced specific viscosity η_{red} measurements can be used as a simple to use assay for stability of polysaccharide dispersions. Stability of chitosan (CL210) solutions (1 mg/ml in a pH4.0, $I = 0.2$ M aqueous solvent) in response to long-term storage at different temperatures. Decay constants $k = 0.087$ week^{-1} (4°C), 0.317 week^{-1} (20°C), and 0.775 week^{-1} (40°C). From Fee (2005).

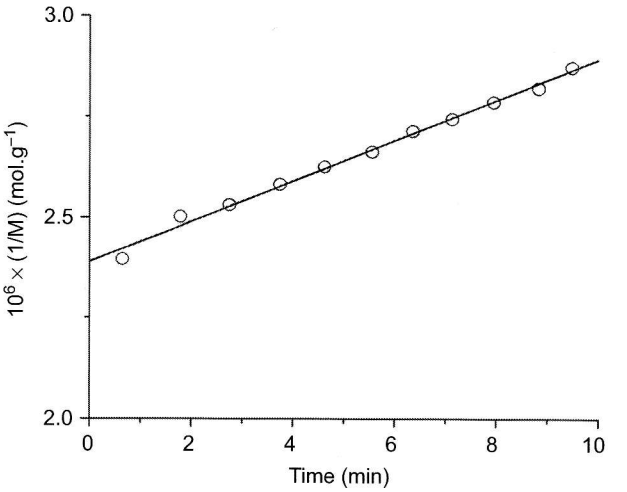

Figure 4. Thermal degradation of κ-carrageenan at 118°C, using a specially adapted slit viscometer. Apparent molecular weights M of 15 mg/ml solutions were estimated using approximate relations from zero shear viscosity measurements. Adapted from Bradley and Mitchell (1988).

as the temperature of measurement was increased from 20°C to 60°C there was no change in η_{red} and no change in M_w. The sedimentation coefficient $s_{T,b}$ (at temperature T and in buffer/solvent conditions b) appeared to increase significantly. However this increase was solely due to a decrease in viscosity and decrease in density of the aqueous solvent. When the sedimentation coefficient $s_{T,b}$ values were corrected to standard conditions of the density and viscosity of water at 20°C to yield $s_{20,w}$ values there was no change. These results clearly showed that across this temperature range the structural integrity of pectin is maintained. A different situation was observed for pectins with a high degree of esterification (Morris et al., 2002). In contrast to the earlier result for the LM pectins, clear degradation was observed for these high methoxyl (HM) pectins: the intrinsic viscosity [η], and molecular weight M all decreased with temp of measurement (Table 1).

Morris et al. (2002) were then able to use the dataset of [η] versus M to see if the slope of a double logarithmic plot remained approximately constant over the range of (thermally degraded) molecular weight: this was indeed the case (Figure 5), yielding an MHKS *a* coefficient of 0.84, consistent with a semi-flexible coil conformation. From this it could be inferred that:

1. thermal treatment led to chain scission of high methoxy pectins
2. there was no noticeable change in conformation from approximately semi-flexible coil
3. from comparison of the earlier study on LM pectin (Morris et al., 1999) the thermal stability of pectins seems strongly correlated with the degree of esterification.

Effects of irradiation on carbohydrate polymers

Another form of sterilization is irradiation by γ- or X-ray radiation (Murray, 1990). Both have sufficient energy to produce ionization of material leading to disruption of the DNA of microbial and other contaminants to products. The disruption is either by direct cleavage of the DNA or secondary cleavage through the release of free-radicals. The construction of γ-irradiation facilities is more cost effective than X-rays and such facilities are available also for the sterilization of foods and hospital equipment. The source is usually ^{60}Co and at no stage do the products come into contact with the radiation source. Dosage is measured in terms of Grays (Gy) where 1 Gy is 1 Joule of energy absorbed by 1 kg of material. Medium doses of up to 10 kGy are normally sufficient to eliminate non-sporulating microorganisms, but higher doses are required if there is a risk of contamination of the product by bacterial spores.

Table 1. Effect of temperature of measurement on the physical properties of high methoxy pectin.

Temperature (°C)	[η] (ml/g)	$10^{-3} \times M_w$ (g/mol)
20	406 ± 2	156 ± 10
30	387 ± 4	145 ± 10
40	362 ± 4	133 ± 10
50	338 ± 3	127 ± 10
60	321 ± 8	117 ± 10

Adapted from Morris et al. (2002).

Irradiated starch

The first study on the effects of irradiation on polysaccharides was a food-based study on starches by Greenwood and MacKenzie (1963) who measured the effect of γ-irradiation dose (up to 100 kGy) on the intrinsic viscosity of potato amylose, an α(1–4) linked glucan. A dramatic decrease was observed with increase in dose, which these researchers interpreted in terms of decrease in degree of polymerization. To make this interpretation they first of all assumed values for K′ and *a* in the MHKS relation (eqn 1) to convert intrinsic viscosities to molecular weights, M, and then the degree of polymerisation *p* is simply M/162, where 162 g/mol is the molar mass of a glucose residue (Table 2).

Jumel et al. (1996) also used intrinsic viscosity measurements of [η] but reinforced by size exclusion chromatography–multiangle laser light scattering (SEC-

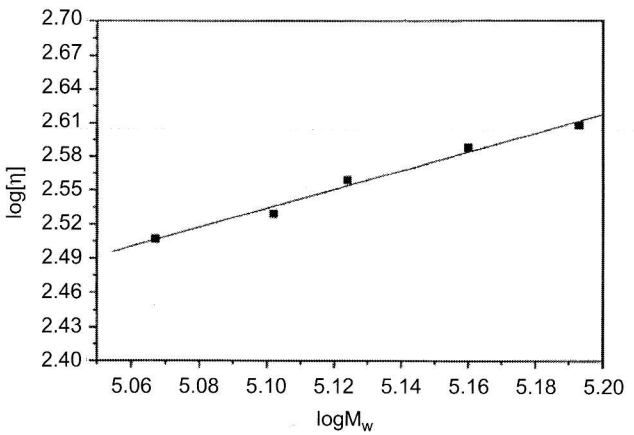

Figure 5. MHKS plot for HM pectin. Plotting a dataset of intrinsic viscosity against molecular weight of the thermally degraded HM pectins no trend is observed in the slope, *a*, of the double logarithmic plot. $a \sim 0.84$, commensurate with a flexible coil conformation.

Table 2. Effect of γ-irradiation dose on amylose.

Dose (kGy)	Intrinsic viscosity [η] ml·g^{-1}	Degree of polymerization *p*
0	230	1700
0.5	220	1650
1	150	1100
2	110	800
5	95	700
10	80	600
20	50	350
50	40	300
100	35	250

Adapted from Greenwood and MacKenzie (1963).

MALLS) measurements of the weight average molecular weight M_w and molecular weight distribution, and with sedimentation coefficient measurements using the analytical ultracentrifuge to evaluate the effects of γ-irradiation on the macromolecular integrity of guar, a galactomannan with a β(1–4) linked mannan backbone with α(1–6) linked galactose side chains. M_w values for the guar in response to level of irradiation (up to 9 kGy) showed a 5 fold drop (Table 3) and the distributions of molecular weight became correspondingly broader (Figure 6). On the basis of the molecular weight data these researchers defined an index for quantifying the incremental degree of disruption called the scission index $G_{scission}$:

$$G_{(\text{scissions})} = \frac{S_{1000} \times 100}{\text{dose}\left(\text{eV}\cdot\text{g}^{-1}\right) \times \text{g}\left(1000\,\text{bonds}\right)^{-1}} \quad (2)$$

where 1 Gy = 6.24×10^{15} eV·g^{-1}. S_{1000} is the number of scissions per 1000 glycosidic bonds, defined by $S_{1000} \sim 1000\{p_o^{-1} - p^{-1}\}$ where p_o is the degree of polymerization of non-irradiated guar. Table 3 shows the incremental G values as the dose is increased. It can be seen that as the chains become shorter and shorter, the chains become more difficult to break.

Table 3. Effect of γ-irradiation dose on guar.

Dose (kGy)	$10^{-6} \times M_w$ g·mol^{-1}	Intrinsic viscosity [η] ml·g^{-1}	Scission index $G_{scission}$
0	2.70	1576	—
0.11	2.03	1467	10.34
0.20	2.32	1360	2.96
0.37	1.78	1360	5.00
0.50	1.87	957	3.14
0.65	1.66	1092	3.37
0.86	1.49	894	3.40
1.70	1.24	964	2.48
5.07	0.866	736	1.49
9.07	0.565	471	1.48

Adapted from Jumel et al. (1996).

Sedimentation velocity experiments in the analytical ultracentrifuge showed the degradation was uniform, with the sedimentation diagrams showing single unimodal traces for the irradiated samples, and perhaps unsurprisingly an increased degree of solubility. A double logarithmic MHKS plot of [η] with M_w yielded an MHKS coefficient $a \sim 0.73$, consistent with other estimates for guar of a very flexible conformation (Picout et al., 2001; Patel et al., 2006; Morris et al., 2008). The conclusion was that for guar, although irradiation produces significant chain scission, it seems to have little effect on chain conformation, at least below 10 kGy.

Irradiated xyloglucans

A more recent study by Patel et al. (2008), again using analytical ultracentrifugation, SEC-MALLS and viscometry has shown similar effects for xyloglucan up to a dose of 70 kGy. This is a β(1–4) linked glucan with α(1–6) linked xylose single residue side chains sometimes capped with galactomannan. Composition analysis indicated that irradiation dose did not significantly alter the ratio of glucose to xylose and galactose (Table 4).

Molecular weight, intrinsic viscosity, and sedimentation coefficient all showed significant reductions with increase in dosage, although unlike with guar there seems to be no noticeable increase in the polydispersity (Table 5).

As with guar, it is possible to use the datasets for intrinsic viscosity and molecular weight together to assess the conformation using the MHKS type of relation, and doing so Patel et al. obtained a value of 0.55 ± 0.03, within the expected range for a random coil (0.5–0.8). Furthermore, the sedimentation coefficient—molecular weight dataset allowed a further power-law type of analysis,

$$s = K''M^b \quad (3)$$

yielding an estimate for b of 0.42 ± 0.01, again between the limits for a random coil (0.4–0.5). All three datasets for M_w, [η] and $s^o_{20,w}$ could be combined into a recently developed global or HYDFIT plot procedure which

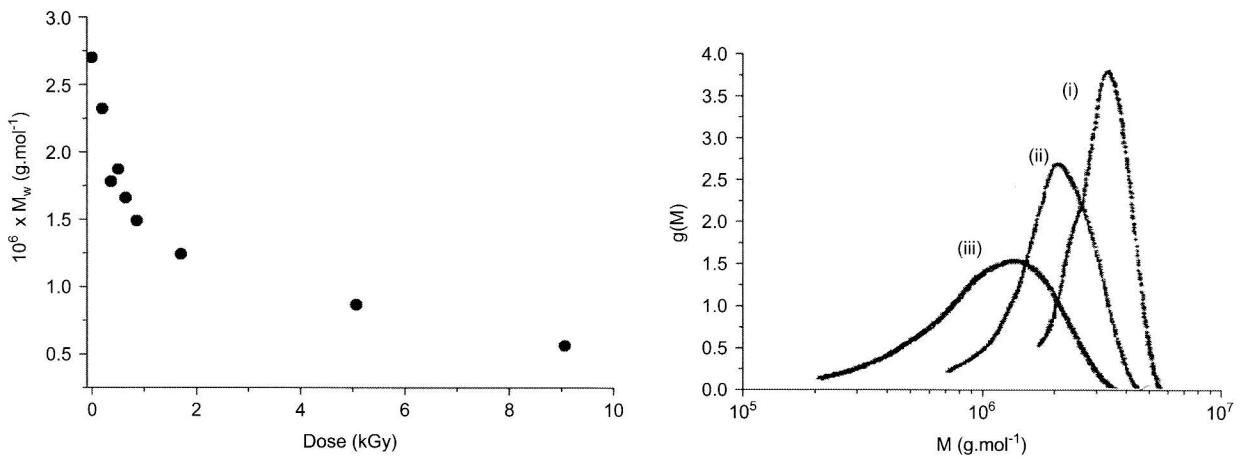

Figure 6. γ-irradiation of guar. (A) Weight average molecular weights fall dramatically and (B) the distributions also become correspondingly broader (i) non-irradiated guar; (ii) 0.20 kGy irradiated; (iii) 1.70 kGy. Adapted from Jumel et al. (1996).

minimizes a target function for best values of the mass per unit length M_L and chain persistence length L_p (Ortega and García de la Torre, 2007). Figure 7 shows such a minimization procedure, yielding a value for L_p (representing the average L_p across the range of dosages) of approximately (7 ± 1 nm) consistent with a flexible coil model (the practical limits are ~2 nm for the highly flexible pullulan and ~200 nm for a stiff rod like conformation like the triple helical polysaccharide schizophyllan). Using a value for the mass per unit length it is then possible to estimate the L_p values of each xyloglucan as a function of dose (Table 5) and it is clear that again, as with guar, despite chain scission there is little change in the conformational flexibility of the chain, with individual values deviating little across the whole range of molecular weight.

Polysaccharide and glycoconjugate vaccines

Polysaccharides are not only used as mucoadhesives, hydrogels, or biofilms but also as direct therapeutic agents themselves. Many have bioactive immunostimulatory properties and appear to underpin the usefulness of many wound healing and other traditional medicines. Polysaccharides and glycoconjugate vaccines against serious disease such as meningitis are becoming increasingly available: again issues of stability are highly important, particularly as many of the active macromolecules are of very high molecular weight (in some cases >100 × 10^6). Again, these substances are substances are subject to the potentially deleterious effects of bioprocessing—including the conjugation process itself—and the physical integrity is as important as considerations of chemical integrity—as assayed by for example mass spectroscopy and ^{1}H and ^{13}NMR (see e.g. Suker et al., 2004). Unfortunately these molecular weights are too large for measurement by SEC-MALLS techniques. In this regard, a recent extension of a sedimentation velocity method applied over two decades ago to the characterization of the molecular weight distribution of mucins (Harding, 1989) should prove useful. The method, originally given by Fujita (1962) for random coils has recently been extended to general conformation types (Harding et al., 2010) and an example is given for a glycoconjugate vaccine characterization in Figure 8.

Table 4. Effect of γ-irradiation dose on the monosaccharide composition of xyloglucans.

Dose (kGy)	Glc-Xyl-Gal (mole ratios)	Xyl:Gal
0	1:0.68:0.32	2.1:1
10	1:0.64:0.31	2.1:1
20	1:0.63:0.31	2.0:1
30	1:0.66:0.31	2.1:1
40	1:0.64:0.32	2.0:1
50	1:0.60:0.32	1.9:1
70	1:0.78:0.36	2.2:1

Adapted from Patel et al. (2008).

Concluding remarks

The considerable developments in methodology that have been made in recent years now provide the Biopharma Industry with the necessary tools to assess

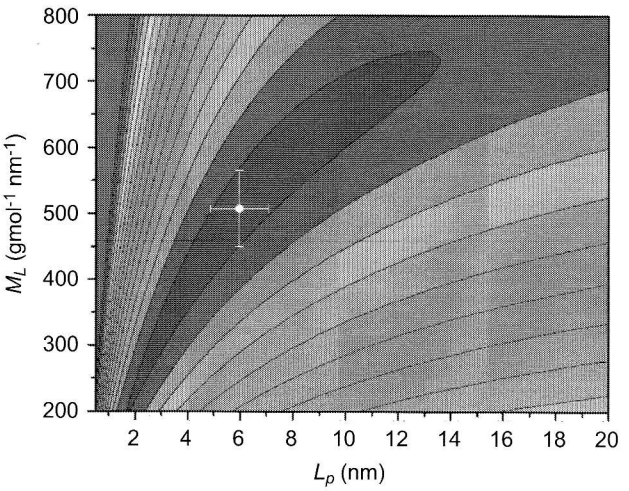

Figure 7. Global or HYDFIT analysis of hydrodynamic data sets of intrinsic viscosity, sedimentation coefficient. Minimisation of a target function (the bluer the colour the lower the function) yields the best estimates for the persistence length L_p and mass per unit length M_L. For the xyloglucans L_p =(7 ± 1) nm consistent with a flexible coil model. From Patel et al. (2008).

Table 5. Effect of γ-irradiation dose on the physical properties of xyloglucans.

Dose (kGy)	$10^{-3} \times M_w$ g·mol^{-1}	Polydispersity M_w/M_n	Intrinsic viscosity [η] ml·g^{-1}	Sedimentation coefficient $s^o_{20,w}$ (S)[a]	Persistence length L_p (nm)
0	700 ± 5	1.1 ± 0.1	405 ± 35	7.21 ± 0.03	6 ± 1
10	270 ± 10	1.3 ± 0.1	210 ± 10	4.66 ± 0.03	6 ± 1
20	158 ± 3	1.4 ± 0.1	170 ± 10	3.10 ± 0.04	9 ± 1
30	127 ± 10	1.3 ± 0.1	140 ± 10	3.30 ± 0.01	6 ± 1
40	97 ± 10	1.3 ± 0.1	135 ± 5	2.82 ± 0.04	8 ± 1
50	60 ± 4	1.3 ± 0.1	100 ± 5	2.80 ± 0.08	6 ± 1
70	45 ± 3	1.1 ± 0.1	75 ± 5	2.61 ± 0.02	6 ± 2

Adapted from Patel et al. (2008).

[a]The superscript "o" means extrapolated to zero concentration to eliminate the effects of non-ideality. The subscript 20,w means the sedimentation coefficient has been corrected or normalized to standard conditions, namely the density and viscosity of water at 20°C. S is the Svedberg unit = 10^{-13} s.

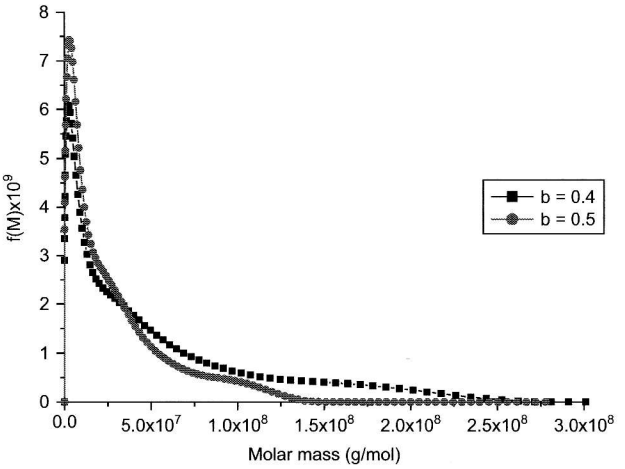

Figure 8. Molecular weight distribution for a large glycoconjugate vaccine construct of a protein and bacterial polysaccharide obtained from sedimentation velocity data and the method described in Harding et al. (2010). Loading concentration c_0 ~0.3 mg/ml. The method requires an approximate idea of the overall conformation and distributions for two reasonable selections of the power-law coefficient b are shown.

the physicochemical stability of macromolecular delivery systems—and macromolecular-based therapeutic agents themselves—in terms of structural integrity: the robustness of products against the stresses of bioprocessing, in terms of aggregation (a particular problem for protein-based systems) and chain degradation (a particular problem for saccharide-based systems) can now be quite precisely assessed, even for very large glycoconjugate assemblies. Subtle chemical structural changes can have significant affects in this regard—as has been demonstrated from a single change to an amino acid in a monoclonal antibody improving its resistance to aggregation after freeze thaw, and varying the degree of esterification of pectins had a dramatic affect on stability against thermal degradation.

Although degradation of polysaccharides appears to be not commensurate with an alteration of the general conformation state (as manifested by the MHKS parameters or the chain persistence length), intriguingly with monoclonal antibodies there is an indication of a conformation link to the aggregation process: this is the subject of current research.

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Declaration of interest

There are no conflicts of interest.

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